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(54) Title: DIAGNOSIS OF MIGRAINE WITH AURA, DEPRESSION AND ANXIETY FROM ALLELIC VARIATIONS IN DOPAMINERGIC GENES

(57) Abstract

The invention provides methods of diagnosing a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety. Diagnosis entails detecting a variant form of one or more dopaminergic genes, such as DRD1, DRD2, DRD3 and DAT. Methods of screening for therapeutic agents effective to treat the syndrome and methods of treatment are also provided.

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DIAGNOSIS OF MIGRAINE WITH AURA, DEPRESSION AND ANXIETY FROM ALLELIC VARIATIONS IN DOPAMINERGIC GENES

CROSS REFERENCE TO RELATED APPLICATIONS

This application derives priority from 60/024,399, filed August 22, 1996 and 60/036,091, filed January 17, 1997, which are incorporated by reference in their entirety for all purposes.

TECHNICAL FIELD

The present invention relates generally to the diagnosis and treatment of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety.

BACKGROUND

Migraine headaches are a type of vascular headaches. Migraine headaches are characterized in part as recurrent attacks of headaches, with or without associated visual and gastrointestinal disturbances. Symptoms of migraine headaches usually follow a pattern in each patient. Attacks may be daily or only once in several months. Untreated attacks may last for hours or even days. Nausea, vomiting, photophobia and sonophobia are common. The extremities can become cold and cyanosed, and the patient can become irritable and seek seclusion. In the United States alone, approximately 18 million females and 5.7 million males have been estimated to suffer from severe migraine annually. Migraines are believed to be a leading cause of lost time from the workplace.

A classification of migraines based on patient

symptoms has been proposed by the Committee on the Classification of Headache of the International Headache Society, *Cephalalgia* 8:1-96 (1988). The committee proposes that migraines be classified a migraine without aura (formerly known as common migraine), migraine with aura (formerly known as classic migraine) and hemiplegic migraine (formerly known as complicated migraine). Migraine with aura and migraine without aura are the two most frequent forms of migraine.

A locus for familial hemiplegic migraine has been reported to occur on chromosome 19, Joutel *et al.*, *Nature Genetics* 5, 40-45 (1993); Joutel *et al.*, *Am. J. Hum. Genet.* 55, 1166-1172 (1994).; Ophoff *et al. Genomics* 22, 21-26 (1994); Elliott *et al.*, Ann. Neurol. 39, 100-106 (1996)). However, the genetic bases and corresponding biochemical mechanisms underlying other forms of migraine disease, such as migraine with aura and migraine without aura, have not been reported and it is unknown whether the different types of migraine are different in kind or only in degree.

At present no specific genetic or biochemical tests are available for the positive diagnosis of migraine with or without aura. Diagnosis and treatment is presently based solely on patient self-reporting and symptom description. Although a wide variety of agents, such as anti-inflammatory drugs, ergots, 5-HT_1 receptor agonists and antiemetics, have been reported to have some benefit for treatment of migraine, their use is complicated by the clinical heterogeneity associated with migraine, side effects of drugs, and the limitations of patient reporting (Caviness *et al.*, *N. Engl. J. Med.* 302, 446-450 (1980); Wilkinson, *Cephalalgia* 3, 61-67 (1983); Peatfield, *Handbook of Clinical Neurology* (ed Rosem Raven Press, New York, 1986) pp. 173-216; Welch, *N.E.J.M.* 329, 1476-1483 (1993); Peroutka, *The Pharmacological Basis of Therapeutics* (eds. Hardman et al., McGraw-Hill, New York, 1996) pp. 487-502. For example, there have been several reports that antiemetics such as domperidone, prochlorperzine, and metoclopramide have beneficial

effects in some migraine patients. However, because the subtype of migraine patient in which such drugs may be effective is not known and because possible side effects of these drugs mitigate against administration without a clear expectation of benefit, these drugs have not been widely administered and have not been approved by the FDA for treatment of migraine. Thus, it has been concluded that, at present, migraine is neither efficiently diagnosed nor managed (*Cephalalgia* 8, 96 (1988)).

Similarities exist between the epidemiological characteristics of migraine, anxiety and depression (Robins et al., *Arch. Gen. Psychiatry* 41:949-958(1984); Stewart et al., *JAMA* 267:64-69 (1992); Rasmussen & Eisen, *J Clin Psychiatry* 55:5-14 (1994); Kessler et al., *Arch. Gen. Psych.* 51:8-19 (1994), Marazziti et al., *Biol. Psychiatr.* 31:125-129 (1995). All three disorders afflict approximately 10 to 25% of the general population at some point in life and are approximately twice as common in females than males. Prophylactic medications for all three disorders have a subacute onset of action, requiring 3 to 6 weeks of therapy to measure clinical improvement.

Identification of inheritance pattern(s) and genetic bases for migraine, depression and anxiety would greatly facilitate the diagnosis and treatment of these diseases. The present invention fulfills this and other needs.

SUMMARY OF THE INVENTION

In one aspect, the invention is directed to methods of diagnosing a patient for susceptibility to a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety. The methods entail detecting a variant allele of one or more dopaminergic genes in the patient. Dopaminergic genes correlated with the syndrome include DRD1, DRD2, DRD3 and DAT. For example, the presence of homozygous A1 alleles of the DRD2 NcoI A1 gene indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates

increased susceptibility relative to the presence of homozygous A2/A2 alleles. In some methods, variant alleles are detected in two or more of the DRD1, DRD2, DRD3 and DAT, and risk factors associated with the presence of each variant allele detected are combined to indicate susceptibility to the syndrome.

The invention further provides methods of treating a patient suffering from the syndrome described above. The patient is administered a therapeutically effective amount of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT. Some such agents lack specific binding to DRD4 and/or DRD5. Exemplary agents are shown in Table 1. Some agents are incapable of permeating the blood-brain barrier. Agents can be administered intravenously, orally or intramuscularly. Agents can be administered therapeutically or prophylactically. Patients amenable to treatment with such methods include those suffering from migraine with aura and having homozygous DRD2 NcoI A1 alleles.

In another aspect, the invention provides methods of screening for a drug effective to treat the syndrome described above. Such drugs are screened by determining their capacity to antagonize binding of dopamine to a dopaminergic receptor or DAT. Optionally, such drugs can be screened for lack of specific binding to DRD4 and/or DRD5.

In another aspect, the invention provides for the use of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT for the manufacture of a medicament for use in the treatment of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety in patients having a variant allele of one or more dopaminergic genes. Such dopaminergic genes include DRD1, DRD2, DRD3 and DAT. Some suitable agents are listed in Table 1. For some agents, the blood-brain barrier is impermeable to passage of the agent. Some agents antagonize DRD1, DRD2, DRD3 and/or DAT without binding to DRD4 or DRD5. In some

uses, the variant allele is DRD2 NcoI A1. The presence of homozygous A1 alleles indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A2/A2 alleles. Thus, a patient having homozygous DRD2 NcoI A1 alleles has a relatively high susceptibility to the syndrome. In some patients, the syndrome is manifested by symptoms of migraine with aura. In some of the uses, the medicament is administered intravenously, orally or intramuscularly. In some uses noted above, the medicament is administered prophylactically.

In another aspect, the invention provides methods for determining the suitability of a patient suffering from a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety for treatment with an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT. The methods entail detecting a variant allele of one or more dopaminergic genes in the patient. Some exemplary agents are listed in Table 1. For some agents, the blood-brain barrier is impermeable to passage of the agent. Some agents antagonize DRD1, DRD2, DRD3 and/or DAT without binding to DRD4 or DRD5. Such dopaminergic genes include DRD1, DRD2, DRD3 and DAT. In some methods, the variant allele is DRD2 NcoI A1 and the presence of homozygous A1 alleles indicates increased suitability relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A1/A2 alleles. Thus, a patient having homozygous DRD2 NcoI A1 alleles has a relatively high susceptibility to the syndrome. In some patients, the syndrome is manifested by symptoms of migraine with aura. In some methods, the agent is administered intravenously, orally or intramuscularly.

The invention further provides for use of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT for the manufacture of a medicament for

therapy. In such uses, a patient is diagnosed for susceptibility to a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety by a method comprising the step of detecting a variant allele of one or more dopaminergic genes in the patient. Often, the one or more dopaminergic genes for which a variant allele is detected are selected from DRD1, DRD2, DRD3 and/or DAT. In some such uses, the variant allele is DRD2 NcoI A1 and the presence of homozygous A1 alleles indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A2/A2 alleles. Thus, a patient having homozygous DRD2 NcoI A1 alleles has a relatively high susceptibility to the syndrome. In some patients, the syndrome is manifested by symptoms of migraine with aura. After diagnosis of the patient, if appropriate, a therapeutically effective amount of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT is then administered to the patient. Some exemplary agents are listed in Table 1. For some agents, the blood-brain barrier is impermeable to passage of the agent. Some agents antagonize DRD1, DRD2, DRD3 and/or DAT without binding to DRD4 or DRD5 In some of the above uses, the agent is administered intravenously, orally or intramuscularly. In some uses, the agent is administered prophylactically.

The invention further provides diagnostic agents (e.g., allele-specific probes and primers), for detecting a variant allele of one or more dopaminergic genes for use in therapy, prophylaxis or diagnosis. Such dopaminergic genes can be selected from DRDI, DRD2, DRD3 and DAT. For example, some diagnostic reagents are used to detect the NcoI A1 allele of DRD2.

The invention further provides agents, such as any of those described above, for use in therapy, prophylaxis or diagnosis of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety. The

invention further provides for use of such agent in the therapy, prophylaxis or diagnosis of migraine with aura.

The invention further provides for the use of an agent for detecting a variant allele of one or more dopaminergic genes for the manufacture of a diagnostic for use in therapy, prophylaxis or diagnosis. Preferred dopaminergic genes are selected from DRD1, DRD2, DRD3 and DAT. For example, a suitable variant allele is DRD2 NcoI A1. The diagnostic is typically used in the therapy, prophylaxis or diagnosis of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety. Further, the diagnostic can be used in the therapy, prophylaxis or diagnosis of migraine with aura.

The invention further provides for the use of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT for the manufacture of a medicament for use in the treatment of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety associated with the presence of a variant allele of one or more dopaminergic genes. In some such uses, a variant allele is present in one or more dopaminergic genes selected from DRD1, DRD2, DRD3 and DAT. In some such uses, the variant allele is DRD2 NcoI A1 and the presence of homozygous A1 alleles indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A2/A2 alleles. Thus, a patient having homozygous DRD2 NcoI A1 alleles has a relatively high susceptibility to the syndrome. In some patients, the syndrome is manifested by symptoms of migraine with aura. Some exemplary agents are listed in Table 1. For some agents, the blood-brain barrier is impermeable to passage of the agent. Some agents antagonize DRD1, DRD2, DRD3 and/or DAT without binding to DRD4 or DRD5. In some uses, the agent is administered intravenously, orally or intramuscularly. In some uses, the agent is administered prophylactically.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-1D show the percentage of individuals having migraine with aura for different DRD1, DRD2, DRD3 and DAT alleles.

Figure 2 shows a risk factor analysis of migraine with aura.

DETAILED DESCRIPTION

I. General

The present invention provides methods of diagnosing and treating a syndrome (mada) characterized by symptoms of, or susceptibility to, migraine with aura, depression and/or anxiety, and resulting, at least in part, from variation in one or more dopaminergic genes. These methods are premised in part on the insight that variations in the dopaminergic genes DRD1, DRD2, DRD3 and DAT are associated with increased susceptibility to migraine with aura, depression and/or anxiety.

DRD1, DRD2 and DRD3 are three of five G proteincoupled receptors (DRD1-DRD5) for which dopamine appears to be the primary neurotransmitter. O'Dowd et al., "Dopamine Receptors," *in Handbook of Receptors and Channels* (ed. Peroutka, CRC Press, Boca Raton, 1994). DRD1 is encoded by an intronless gene (Dearry *et al.*, *Nature* 347:72-76 (1990); Zhou *et al.*, *Nature* 347:76-80 (1990); Sunahara *et al.*, Nature 347:80-83 (1990)) and is expressed most abundantly in the caudate, nucleus accumbens and olfactory tubercle. DRD1 receptors are thought to act as pre-synaptic autoreceptors modulating neurotransmitter release.

The DRD2 gene has a length of about 270 kb, including six introns, the first of which accounts for about 200 kb of the gene. A cDNA sequence of 2500 bp has been reported, which includes some flanking sequences. Grandy et al., *Proc. Natl. Acad. Sci.* 86, 9762-9766 (1989). DRD2s are localized in numerous anatomical locations that are believed to play a major role in the pathogenesis of migraine. The highest density of DRD2s

are in the substantia nigra and basal ganglia. In the substantia nigra, the DRD2s act as presynaptic autoreceptors which modulate dopamine release (Mengod *et al., Neurochem. Int.* 20, 33S-43S (1992)). In addition, dopamine receptors have been located directly in vascular beds (e.g., on cerebral arteries) that are believed to be critical in the pathogenesis of migraine. For example, dopamine receptors have been localized to pial vessels (Oudart *et al., Arch. Int. Pharmacodyn.* 252, 196-209 (1981); Edvinsson *et al., Br. J. Pharmac.* 85, 403-410 (1985)) the site of neurogenic inflammation that is believed to play a major role in the headache component of migraine (Moskowitz, *Trends Pharmacol. Sci.* 13, 307-311 (1992)). DRD2s are also located in the peripheral and/or central sympathetic nervous system. DRD2s are also located on presynaptic noradrenergic sympathetic ganglia, where they act to inhibit the release from the sympathetic nerve terminals (Clark *et al., Acta Endocrine., Suppl.* 216 88, 75-81 (1978); Ziegler *et al., Clin. Pharmacol. Ther.* 25, 137-142 (1979); Mercuro, *Eur. J. Clin. Pharmacol.* 27, 671-675 (1985); Montastruc *et al. Eur. J. Pharmacol.* 166, 511-514 (1989)).

The DRD3 gene has a high degree of sequence identity with the DRD2 gene (O'Dowd *et al., Handbook of Receptors and Channels: G Protein-coupled Receptors* (ed Peroutka, S.J.) 95-123 (CRC Press, Boca Raton, 1994); Sokoloff *et al., Nature* 347:146-151 (1990)). Like DRD2, the DRD3 receptor acts as both a postsynaptic receptor and an autoreceptor which inhibits dopamine release (Tang *et al., J. Pharmacol. Exp. Ther.* 270:475-476 (1994)). Expression of DRD3 has been localized to the limbic areas of the brain (Mengod *et al., Neurochem. Int.* 20:33S-43S (1992); Sokoloff *et al., Nature* 347:146-151 (1990)) suggesting that it may be associated with cognitive, emotional and endocrine functions. Most drugs that interact with DRD2 also interact with similar affinity with DRD3 (Sokoloff *et al., Nature* 347:146-151 (1990)). However, the density of expressed DRD3 receptors is low,

estimated at about 1% of that of DRD2 (Accili *et al.*, *Proc. Nat. Acad. Sci.* 93:1945-1949 (1996)).

The dopamine transporter (DAT) gene is a key regulatory protein in the dopamine pathway that modulates the amount of dopamine release and re-uptake (Shimada *et al.*, *Science* 254:576-577 (1991)).

Variant alleles of the dopaminergic genes DRD1, DRD2, DRD3 and DAT probably cause increased susceptibility to migraine with aura, depression and/or anxiety as a result of increased dopaminergic transmission. The proposed role of dopaminergic genes in migraine with aura is consistent with several clinical features of the disease. For example, nausea and/or vomiting are common features of migraine in which dopamine stimulation is likely. Gastrokinetic changes, hypotension and other autonomic nervous system changes are additional migraine symptoms that are consistent with disturbances in dopaminergic neurotransmission. The proposed mechanism is also consistent with previous reports of exaggerated autonomic responses to dopamine agonists in migraine patients. For example, apomorphine has been reported to induce symptoms of migraine, as well as the associated phenomenon of nausea, vomiting, yawning, hypotension and syncope (DelZompo et al., *Headache* 35, 222-224 (1995)).

II. Analysis of Polymorphisms in Dopaminergic Genes

The methods of diagnosis and treatment described below usually require knowledge of the genotype of an individual with respect to polymorphisms in the genes DRD1, DRD2, DRD3, and/or DAT. Exemplary polymorphisms within each of these genes that correlate with migraine with aura, depression and/or anxiety are described in the Examples, as are methods for their detection. Specifically, these polymorphisms include: an A to G polymorphism in the 5' untranslated region of DRD1 having alleles designated B1 and B2 as described by Cichon *et al.*, *Hum. Mol. Genet.* 3:209-209 (1994); a C to T

polymorphism at codon 313 of DRD2 having alleles designated NcoI A1 and NcoI A2 (Sarker *et al.*, *Genomics* 11:8-14 (1991)); an A to G substitution 25 bp downstream from the start codon in DRD3 having alleles designated A1 and A2 (Rietschel *et al.*, *Psychiatr. Res.* 46:253-259 (1993)); and a polymorphic 40 bp repeat in the 3' untranslated region of DAT having alleles designated 9 and 10 (Vandenbergh et al., *Genomics* 14:1104-1106 (1992)). It is expected that the DRD2 TaqI A1 allele (Noble, *Science & Medicine* 3, 52-61 (1996)), which is known to be in linkage disequilibrium with DRD2 NcoI A1, can be used in diagnostic methods in a similar manner to DRD2 NcoI A1.

Other variant forms of the DRD1, DRD2, DRD3 and DAT genes that correlate with migraine with aura, anxiety and/or depression can be identified as follows. The first step is to identify additional polymorphic sites within one of these genes. Such polymorphic sites can be identified either by comparative sequencing of these genes in a population of individuals or from the published literature and databases. For example, several additional polymorphic sites in the DRD2 gene have been published including TaqIB within intron 2, FokI B at position 1105, HphI at position 3208, C311S at position 3413, NcoI at position 3420 and TaqIA within the 3' untranslated region. (Residues in genomic DNA are assigned the same number as the corresponding nucleotide in cDNA when the two are maximally aligned, and nucleotides in the cDNA are numbered according to the convention of Dal Toso, *EMBO J.* 8, 4025-4034 (1989)). Having identified the location of a polymorphism and the nature of its polymorphic forms, a correlation is performed between type of polymorphic form and presence or absence of migraine with aura, depression, and/or anxiety in a population. Optionally, the correlation can be determined with respect to combinations of two or more polymorphisms within the same gene. For example, individuals having the NcoI A1 allele are subdivided into two classes respectively having A1 and A2 alleles of the

FokI polymorphism. Thus, for example, one can determine whether the NcoI A1/ FokI A1 genotype correlates significantly more strongly with migraine with aura than the NcoI A1/FokI A2 genotype.

Variant genes can be detected, for example, by sequencing, allele-specific amplification (Gibbs, *Nucleic Acid Res.* 17, 12427-12448 (1989)), restriction enzyme analysis, allele-specific probe hybridization assays (Saiki et al., *Nature* 324, 163-166 (1986)) or singlestranded conformational analysis (Orita et al., *Proc. Natl. Acad. Sci.* 86, 2766-2770 (1989)). For example, the NcoI A1 and A2 alleles can be distinguished by NcoI digestion. Only the A2 allele is cut. Reagents used for detecting variant alleles, such as allele specific probes and primers can be packaged as diagnostic reagents. The diagnostic reagents can bear labels indicating their suitability for use in diagnosis of the mada syndrome or a symptom thereof.

III. Susceptibility Analysis

The present data indicate that analysis of DRD1, DRD2, DRD3 and/or DAT gene in a patient can be used as a measure of susceptibility to migraine with aura, depression and/or anxiety. For example, detection of one or both copies of the NcoI A1 allele of DRD2 indicates increased susceptibility to migraine with aura, depression and/or anxiety in the patient, and detection of both copies indicates increased susceptibility relative to one copy. Detection of one or both copies of DRD1 B1 indicates increased risk of migraine with aura relative to homozygous DRD1 B2. Detection of homozygous DRD3 A2 indicates increased risk of migraine with aura relative to homozygous or heterozygous DRD3 A1. Detection of homozygous DAT 10 indicates increased risk of migraine with aura relative to heterozygous DAT 10/9 and probably 9/9 genotype, although the latter occurs with insufficient frequency to have been included in the present analysis.

Although the probability of an individual having any

one variant allele developing the mada syndrome is low (no more than 20%), the individual probabilities combine in an additive fashion. The presence of each variant allele associated with the mada syndrome can be assigned a risk factor related to the probability, as discussed in the Examples. Fig. 2 shows the percentage of individuals having migraine with aura as a function of number of risk factors present. It can be seen that no individuals without any dopaminergic risk factor have symptoms of the mada syndrome and about 75% of individuals with all five dopaminergic risk factors have symptoms of the mada syndrome. Individuals with 1-4 risk factors show intermediate frequencies of symptoms of the mada syndrome in relation to the number of risk factors present.

There are probably other genes besides the dopaminergic genes described above having variant forms associated with risk of the mada syndrome. The existence of variant forms of such genes can be detected and correlated with probabilities of susceptibility to the syndrome in similar fashion to the analysis of dopaminergic genes. Combined statistical analysis of dopaminergic genes with other genes still further increases the predictive value of the diagnosis.

The analysis is useful in identifying a subset of patients having a common genetic basis giving rise to the mada syndrome. Such patients are amenable to treatment with antagonists of dopaminergic genes as discussed below. Treatment with such antagonists may be ineffective in other patients, who exhibit similar symptoms to patients with the syndrome, but due to a different genetic basis. This analysis is also useful in distinguishing migraine with aura from migraine without aura, and others diseases, such as stroke, which may present with similar symptoms. That is, a patient with a high number of risk factors for the mada syndrome is more at risk for migraine with aura than for migraine without aura or stroke.

IV. Methods of Treatment

The invention further provides methods of treating patients suffering from, or susceptible to, the mada syndrome. In these methods, a patient having or susceptible to symptoms of migraine with aura, depression and or anxiety is treated with a therapeutically effective dose of an antagonist to one or more of the dopaminergic genes DRD1, DRD2, DRD3 and DAT. Such a dose is sufficient to prevent, arrest or detectably relieve symptoms of migraine with aura, depression and/or anxiety. The dose can be administered prophylactically or therapeutically. The dose can also be administered to pediatric or handicapped patients but who are unable to articulate their symptoms but are known to have variant forms of one or more variant forms of the dopaminergic genes associated with the disease.

In general, the stronger the binding of an antagonist to the dopaminergic protein, the greater the efficacy. Buproprion (2-tert-butylamino-3'-chloropropiophenone hydroxide) manufactured by Glaxo Wellcome is an example of a DAT antagonist. A list of DRD2 antagonists and appropriate dosages is provided in Table 1. Many of these antagonists also bind to other dopaminergic receptors, particular DRD3 and DRD4, which are most closely related to DRD2. DRD2 antagonists include phenothiazines (chlorpromazine, fluphenazine, prochlorperazine, promethazine, thioridazine and trifluoperazine), butyrophenomes (droperidol, haloperidol, pimozide, spiperone), thioxanthines (chlorprothixene, thiothixene) and other drugs, such as clozapine. Some antagonists are capable of crossing the blood-brain barrier and therefore capable of antagonizing both central and peripheral dopaminergic receptors. Other antagonists such as domperidone do not cross the blood-brain barrier and therefore antagonize only peripheral receptors. Some agents such as domperidone, metoclopramide, chlorpromazine, prochloperazine and flunarazine have previously been reported to have some value in treating some migraine patients. However, the

mechanism of action was not known, not was it appreciated that the agents are most appropriate for administration to the subset of migraine patients having migraine with aura and variant forms of one or more of the dopaminergic genes DRD1, DRD2, DRD3, DRD4 and DAT.

Antagonists can be used to manufacture medicaments for use in treatment of the syndrome. Antagonists can be mixed with a pharmaceutical carrier, which can be any compatible, non-toxic substance suitable to deliver the antagonist to the patient. Sterile water, alcohol, fats, waxes, and inert solids can be used as the carrier. Pharmaceutically-acceptable adjuvants, buffering agents, dispersing agents, and the like, can also be incorporated into the pharmaceutical compositions. The concentration of the active agent in the pharmaceutical composition can vary widely, i.e., from less than about 0.1% by weight, usually being at least about 1% by weight to as much as 20% by weight or more. Medicaments can be administered intravenously, intramuscularly, subcutaneously, intranasally, cutaneously, via suppository, by inhalation or orally. Methods for preparing parenterally administrable compositions are described in more detail in, for example, *Remington's Pharmaceutical Science* (15th ed., Mack Publishing Company, Easton, Pennsylvania, 1980) (incorporated by reference in its entirety for all purposes).

For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. Active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium

lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

V. Screening Drugs

The invention further provides methods of screening for novel antagonists of DRD1, DRD2, DRD3 and/or DAT for treatment of the syndrome. Potential agents are screened for specific binding (Kd < μM) to human DRD1, DRD2, DRD3 or DAT, optionally in competition with dopamine. Preferred agents bind with a Kd less than 10 nM and can therefore usually be used at a dose of about 10 mg/patient. Receptor binding assays can be performed as described by Ison & Peroutka, *Cancer Treatment Reports* 70, 637 (1986); Peroutka & Snyder, *Am. J. Psychiatry* 137, 12 (1980) using cloned and expressed human receptors or human receptors located in post-mortem human tissues. Some agents are screened for lack of specific binding to at least one of dopaminergic receptors DRD4 or DRD5. The agents can also be screened for lack of specific binding to other receptors to minimize side effects. For example, lack of binding to the α-adrenergic receptor minimizes orthostatic hypotensive side-effects. Preferred agents have a serum half-life of about 24 hr and can reach peak plasma levels within about 15-60 min of administration.

EXAMPLES

Correlations Between Migraine with Aura and Dopaminergic Genes

This example describes analysis of allelic variants within all five dopamine receptor genes (DRD1, DRD2, DRD3, DRD4 and DRD5) and the DAT gene in control, migraine without aura (MO) and MWA individuals.

A. METHODS

1. Subjects

Subjects were identified for this study by physician referral. Individuals were evaluated using the diagnostic criteria for MO and MWA established by the International Headache Society (*Cephalalgia* 8, 1-96 (1988)). The lifetime presence or absence was determined for each of the criteria in the IHS definition of migraine. Interviews were conducted by physicians, nurses and/or trained interviewers. All interviewers were trained by the present inventor in the use of the IHS criteria and all clinical data were reviewed by the same neurologist. Control group individuals did not meet IHS criteria for migraine (based on direct interview) and were predominantly unaffected spouses of the individuals with migraine. Informed consent was obtained and DNA samples collected. All clinical data were obtained independently of the genotypic data. The average age of the study participants is 53 $\pm$ 1 years. No variation was observed between the 3 study groups in terms of age, sex or ethnic origin.

2. Genotyping

A total of 246 DNA samples from unrelated individuals, who were 35 years of age or older, were analyzed (115 control individuals; 77 MO individuals; 54 MWA individuals). Genomic DNA was isolated using the Puregene DNA isolation kit (Gentra Systems, Research Triangle Park, North Carolina). Genotypes were scored independently by two individuals blinded to the clinical status.

3. *DRD1, DRD2, DRD3 and DRD5*

DRD1, DRD2, DRD3 and DRD5 were amplified as follows. Briefly, 40 ng of genomic DNA was amplified in 10 μL of a solution containing 1x Perkin Elmer PCR amplification buffer, 400 μM each dNTP, 0.5 U *Taq*Gold polymerase (Perkin Elmer, Foster City, CA) and 1 μM primers. The enzyme was activated with an initial incubation at 94°C for 10 minutes, followed by 14 cycles of amplification with denaturation at 94°C for 20 seconds, annealing at 63°C for 1 minute, elongation at 72°C for 30 seconds with a decrease of 0.5°C and 3 seconds for each annealing step, and an additional 40 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and elongation at 72°C for 1 minute. After amplification, 10 uL of a solution containing 2x restriction enzyme buffer and 4 U of the appropriate restriction enzyme were added directly to the amplification reaction and incubated at 37°C for greater than 4 hours. Digested products were separated on 1.5% SFR agarose gels (Amresco, Solon, OH). Primers used for amplification of each marker, restriction enzymes used to distinguish genotypes and the expected sizes of each allele are listed in Table 2.

TABLE 2

ALLELE IDENTIFICATION METHODS

Locus Restriction	Primer	Sequence Restriction	Allele References	
			Enzyme	Sizes
DRD1	5'D1.0	ATTCAGGGGCTTTCTGGTG	*DdeI*	B1 = 285
	3'5'D1.0	AGCAGGGAATAGGGGTCAGT		B2 = 223
DRD2	DRD2.35	ATCCTGCAGCCATGG	*MscI*	A1 = 450
	360RD2.38	ATTGTCCGGCTTTACC		A2 = 250
DRD3	DRD3.PCR1.1	GCTCTATCTCCAACTCTCACA	*MscI*	A1 = 304
	38DRD3.PCR1.2	AAGTCTACTCACCTCCAGGTA		A2 = 208
DRD4	DRD4.SB.PCR3	GTGCACCACGAAGAAGGG	48-bp repeat	4 = 500
	39DRD4.SB.PCR4	GCTGCTGCTCTACTGGGC		7 = 750
DRD5	DRD5-3'	GGGTTGAGTGAGGAGTTAG	*Eco*571	A1 = 380
	40DRD5.SB.PCR2	GACGTGAATGCAGAGAACTG		A2 = 460
DAT	T3-5Long	TGTGGTGTAGGGAACGGCCTGAG	40-bp repeat	9 = 440
	33T7-3sLong	CTTCCTGGAGGTCACGGCTCAAGG		10 = 480

4. *DRD4*

DRD4 genotypes were assessed by amplifying 50 ng of genomic DNA in 10 μL of 1x ThermoPol buffer (New England Biolabs), 400 uM dNTP, 1 uM of each primer (see Table 2) and 0.2 U Vent (exo$^-$) Polymerase. Amplification consisted of 35 cycles of denaturation at 98 °C for 1

minute and annealing/elongation at 70 °C for 5 minutes. Amplification products were analyzed on 1.2% agarose gels.

5. *DAT*

DAT genotypes were assessed by amplifying 40 ng of genomic DNA in the presence of 0.5 μM of fluorescence-labelled dUTP (Applied Biosystems, Foster City, CA). Reactions progressed through 35 cycles of denaturation at 94 °C for 1 minute and annealing/elongation at 72 °C for 1 minute. Amplified products were analyzed on an ABI 373 sequencer using a 6% polyacrylamide gel. Analysis was performed as described previously (Vandenbergh et al., *Mol. Brain Res.* 15, 161-166 (1992)); Doucette-Stamm et al. *Genet. Epidemiol.* 12, 303-308 (1995)).

B. RESULTS

1. DRD1 5' UTR B1 and B2 Allele Frequencies

An A to G polymorphism has been described in the 5' untranslated region of the DRD1 gene (Cichon et al., *Hum. Mol. Genet.* 3, 209-209 (1994)). In the current dataset (n = 246 individuals), the DRD1 B1 allele frequency is 0.37 and the B2 allele frequency is 0.63. These values are similar to the DRD1 B1 and B2 allele frequencies reported in Caucasians (id.). In the overall dataset, 16% of individuals have the B1/B1 genotype, 41% have the B1/B2 genotype and 43% display the B2/B2 genotype.

An association of MWA and the DRD1 B1 allele is apparent in an analysis of genotype distributions (Table 3). No significant difference is observed in the genotypic distribution between the control group and individuals with MO. The B2/B2 genotype in the dataset is significantly less frequent in individuals with MWA (28%) than in control individuals and individuals with MO (Chi-square = 6.28; $p < 0.006$). MWA is observed in 14% of the B2/B2 individuals, 26% of the B1/B2 individuals and 31% of the B1/B1 individuals (Figure 1A).

TABLE 3

DOPAMINE D1 RECEPTOR POLYMORPHISM FREQUENCIES
IN A SAMPLE OF 246 INDIVIDUALS

	Genotypes						Allele Frequencies	
	B1/B1	(%)	B1/B2	(%)	B2/B2	(%)	B1	B2
Controls (n = 115)	15	(13%)	45	(39%)	55	(48%)	0.33	0.67
MO Subjects (n = 77)	12	(16%)	30	(39%)	35	(45%)	0.35	0.65
MWA Subjects (n = 54)	12	(22%)	27	(50%)	15	(28%)	0.47	.53*,**
TOTAL (n = 246)	39	(16%)	102	(41%)	105	(43%)	0.37	0.63

* Chi-square = 6.71 ($p < 0.01$) vs. control group
**Chi-square = 3.91 ($p < 0.02$) vs. MO group
Chi-square = 8.75 ($p < 0.009$) vs. the combined control and MO group

The DRD1 B1 and B2 allele frequencies were also determined in each subgroup of subjects. Similar allele frequencies are observed in both the control group and individuals with MO. By contrast, individuals with MWA have a significantly greater frequency of the DRD2 B1 allele (0.47) than either the control group (Chi-square = 6.71; $p < 0.01$) or individuals with MO (Chi-square = 3.91; $p < 0.02$).

2. DRD2 *NcoI* A1 and A2 Allele Frequencies

A C to T polymorphism, resulting in a silent mutation at amino acid 313, has been described in the DRD2 gene (Sarkar *et al.*, *Genomics* 11, 8-14 (1991).). In the current dataset, the DRD2 *NcoI* A1 allele frequency is 0.73 and the A2 allele frequency is 0.27. These values are similar to the DRD2 *NcoI* allele frequencies reported in the North American population (id.). In the overall dataset, 54% of individuals have the A1/A1 genotype, 37% have the A1/A2 genotype and 8% display the A2/A2 genotype.

An association of MWA and the DRD2 A1 allele is apparent in an analysis of genotype distributions (Table 4). No significant difference is observed in the genotypic distribution between the control group and individuals with MO. The A1/A1 genotype in the dataset is significantly more frequent in individuals with MWA (69%) than in control individuals and individuals with MO (Chi-square = 5.50; $p < 0.01$). MWA is observed in 5% of the A2/A2 individuals, 17% of the A1/A2 individuals and 28% of the A1/A1 individuals (Figure 1A).

TABLE 4

DOPAMINE D2 RECEPTOR *NcoI* POLYMORPHISM FREQUENCIES IN A SAMPLE OF 246 INDIVIDUALS

	Genotypes						Allele Frequencies	
	A1/A1	(%)	A1/A2	(%)	A2/A2	(%)	A1	A2
Controls (n = 115)	57	(50%)	48	(42%)	10	(9%)	0.70	0.30
MO Subjects (n = 77)	40	(52%)	28	(36%)	9	(12%)	0.70	0.30
MWA Subjects (n = 54)	37	(69%)	16	(30%	1	(2%)	0.83	0.17*,**
TOTAL (n = 246)	134	(54%)	92	(37%)	20	(8%)	0.73	0.27

* Chi-square = 6.45 ($p < 0.01$) vs. control group
** Chi-square = 5.99 ($p < 0.01$) vs. MO group
\# Chi-square = 7.28 ($p < 0.007$) vs. the combined control and MO group

The DRD2 *NcoI* A allele frequencies were also determined in each subgroup of subjects (Table 5). Similar allele frequencies were observed in both the control group and individuals with MO. By contrast, individuals with migraine with aura had a significantly greater frequency of the DRD2 A1 allele (0.83) than either the control group or individuals with migraine without aura.

3. DRD3 A1 and A2 Allele Frequencies

A polymorphism resulting in a glycine to serine substitution at position 9 in the N-terminal part of the DRD3 receptor was analyzed (Lannfelt *et al., Psychiatr. Genet.* 2, 249-256 (1992)). The polymorphism consists of a A to G substitution which is 25 bp downstream from the start codon, creating a restriction site for *BalI*. This polymorphism has been hypothesized to play a role in receptor insertion into the cell membrane (Rietschel *et al., Psychiatr. Res.* 46, 253-259 (1993).

In the overall dataset, the DRD3 A1 allele frequency is 0.62 and the A2 allele frequency is 0.37. These values are similar to the DRD3 allele frequencies reported in previous studies (id.). In the overall dataset, 37% of individuals have the A1/A1 genotype, 50% have the A1/A2 genotype and 12% display the A2/A2 genotype.

No significant difference was observed in the genotypic distribution between the control group and individuals with MO (Table 5). However, the DRD3 A2/A2 genotype in the dataset is significantly more frequent in individuals with MWA (20%) than in control individuals and individuals with MO (Chi-square = 4.32; $p < 0.02$).

MWA is observed in 22% of the A1/A1 individuals, 19% of the A1/A2 individuals and 37% of the A2/A2 individuals (Figure 1C). The DRD3 A2 allele frequencies is increased in MWA compared to both control and MO individuals. However, this difference does not reach statistical significance.

TABLE 5

DOPAMINE D3 RECEPTOR POLYMORPHISM FREQUENCIES IN A SAMPLE OF 246 INDIVIDUALS

	Genotypes						Allele Frequencies	
	A1/A1	(%)	A1/A2	(%)	A2/A2	(%)	A1	A2
Controls (n = 115)	45	(39%)	58	(50%)	12	(10%)	0.64	0.36
MO Subjects (n = 77)	27	(35%)	43	(56%)	7	(9%)	0.63	0.37
MWA Subjects (n = 54)	20	(37%)	23	(43%)	11	(20%)*	0.58	0.42
TOTAL (n = 246)	92	(37%)	124	(50%)	30	(12%)	0.63	0.37

* Chi-square = 4.32 ($p < 0.02$) vs. the combined control and MO group

4. DRD4 Allele Frequencies

The DRD4 gene contains a 48-bp sequence in the third cytoplasmic loop of the receptor that ranges from 2- to 8-fold repeat units (Van Tol *et al.*, *Nature* 358, 149-152 (1992). In the current dataset, genotypes were obtained on 238 of the 246 individuals. The observed allele frequency distribution is similar to the DRD4 allele frequencies reported in the North American population (id.): 2 repeats (n = 38; 0.08 allele frequency), 3 repeats (n = 26; 0.05), 4 repeats (n = 310; 0.65), 5 repeats (n = 1; < 0.01) and 7 repeats (n = 101; 0.21). In the current dataset, 106 individuals (45%) display the most common genotype (i.e., 4/4) whereas 91 individuals (38%) have at least one 7 allele (Table 6).

TABLE 6

DOPAMINE D4 RECEPTOR POLYMORPHISM FREQUENCIES IN A SAMPLE OF 238 INDIVIDUALS

	Genotypes						Allele Frequencies	
	7 allele present	(%)	4, 7 genotype	(%)	7 allele absent	(%)	4 allele	7 allele
Controls (n = 110)	38	(35%)	30	(27%)	72	(65%)	0.70	0.18
MO Subjects (n = 75)	34	(45%)	20	(27%)	41	(55%)	0.57	0.27
MWA Subjects (n = 53)	19	(36%)	13	(25%)	34	(64%)	0.65	0.20
TOTAL (n = 238)	91	(38%)	63	(27%)	147	(62%)	0.65	0.21

No significant difference is observed in the

genotypic distribution between the control group, individuals with MO and individuals with MWA (data on the most common genotypes are summarized in Table 6). MWA is observed in 21% of individuals with a 7 allele and 23% of individuals without a 7 allele. Similar allele frequencies are observed in the control group, individuals with MO and individuals with MWA (Table 6).

5. DRD5 A1 and A2 Allele Frequencies

In the current dataset, the DRD5 A1 allele frequency is 0.68 and the A2 allele frequency is 0.32. These values are similar to the DRD5 allele frequencies reported in the North American population (Sommeret al., *Hum. Genet.* 92, 633-634 (1993)). In the overall dataset, 47% of individuals have the A1/A1 genotype, 43% have the A1/A2 genotype and 10% display the A2/A2 genotype.

No significant difference was observed in the genotypic distribution between the control group, individuals with MO and individuals with MWA (Table 7). MWA is observed in 22% of the A1/A1 individuals, 23% of the A1/A2 individuals and 16% of the A2/A2 individuals. Similar allele frequencies are observed in the control group, individuals with MO and individuals with MWA (Table 7).

TABLE 7

DOPAMINE D5 RECEPTOR POLYMORPHISM FREQUENCIES IN A SAMPLE OF 246 INDIVIDUALS

	Genotypes						Allele Frequencies	
	A1/A1	(%)	A1/A2	(%)	A2/A2	(%)	A1	A2
Controls (n=115)	58	(50%)	45	(39%)	12	(10%)	0.70	0.30
MO Subjects (n=77)	32	(42%)	36	(47%)	9	(12%)	0.65	0.35
MWA Subjects(n=54)	26	(48%)	24	(44%)	4	(7%)	0.70	0.30
TOTAL (n=246)	116	(47%)	105	(43%)	25	(10%)	0.68	0.32

6. DAT 9 and 10 Allele Frequencies

A polymorphic 40 bp repeat in the 3' untranslated region as been identified in the DAT gene (Vandenbergh et al., *Genomics* 14, 1104-1106 (1992)). In the overall dataset, the DAT 9 allele frequency is 0.22, the 10 allele frequency is 0.77 and more rare alleles have a

frequency of 0.01. These values are similar to the DAT allele frequencies reported in previous studies (id.). In the overall dataset, 35% of individuals have the 9/10 genotype and 59% display the 10/10 genotype. The less frequent DAT genotypes polymorphisms are as follows: 9/9 (n = 11), 1/2 (n = 2) and 2/8 (n=1). Since only 14 individuals (5.7%) have these less frequent genotypes, these individuals were not analyzed independently in the present study.

No significant difference was observed in the genotypic distribution between the control group and individuals with MO. An association of MWA and the 10/10 genotypes is observed in the present study (Table 8). MWA is observed in 13% of the 9/10 individuals and 25% of the 10/10 individuals (Figure 1D). The 10/10 genotype frequency is significantly higher in the MWA group (69%) in comparison to the 9/10 genotype frequency in the control subjects (38%; Chi-square = 4.46; $p < 0.02$) and to the MO group (40%; Chi-square = 4.41; $p < 0.02$). A higher level of statistical significance is observed when the 10/10 genotype frequency in the MWA group is compared to the 9/10 genotype in the combined control and MO groups (39%; Chi-square = 6.46; $p < 0.006$).

TABLE 8

DOPAMINE TRANSPORTER POLYMORPHISM FREQUENCIES
IN A SAMPLE OF 246 INDIVIDUALS

	Genotypes					Allele Frequencies		
	9/10	(%)	10/10	(%)	other	(%)	9	10
Controls (n=115)	44	(38%)	65	(57%)	6	(5%)	0.22	0.78
MO Subjects (n=77)	31	(40%)	44	(57%)	2	(3%)	0.22	0.77
MWA Subjects (n=54)	11	(20%)	37	(69%)*,**	6	(11%)	0.21	0.79
TOTAL (n=243)	86	(35%)	146	(59%)	14	(6%)	0.22	0.78

* Chi-square = 4.46 ($p < 0.02$) vs. control group
** Chi-square = 4.41 ($p < 0.02$) vs. MO group
# Chi-square = 6.46 ($p < 0.006$) vs. combined control and MO group

7. Allelic "Risk Factor" Assessment

Risk factor analysis has proven to be an important approach to the assessment and management of cardiovascular disease (Kannel, *Hosp. Pract.* 25, 119-130 (1990); Wilson, *Am. J. Hypertens.* 7, 7S-12S (1994);

Hancock, *Scientific American Medicine* (eds Dale, D. C. & Federman, D. D.) 1-12 (Scientific American, Inc., New York, 1995). Applying the principles of risk factor analysis to MWA, an analysis of the data was performed based on the allelic variants which showed independent associations with MWA. The goal of this analysis was to determine if additive and/or synergistic effects exist between the dopaminergic genes in terms of MWA.

Based on the data in the present study, 5 specific allelic "risk factors" were identified that were associated independently with an increased susceptibility to MWA compared to alternative genotypes at the same molecular location within the dopaminergic genes: the DRD1 B1/B1 or B1/B2 genotype, the DRD2 A1/A1 genotype, the DRD2 A1/A2 genotype, the DRD3 A2/A2 genotype and the DAT 10/10 genotype. Although the strength of the associations varied amongst the different genes, an initial attempt to develop a "risk factor" profile for MWA assigned equal weight to each allelic "risk factor" with a single exception: the DRD2 A1 allele. Individuals with the DRD2 A1/A1 genotype were assigned a risk factor of "2" since the frequency of MWA appeared to be related to the number DRD2 A1 alleles present in an individual (Table 4).

The number of allelic "risk factors" were determined for each individual in the present study (i.e., 0-5 dopaminergic allelic "risk factors" per individual). The frequency of MWA was then determined in each allelic "risk factor" group. As summarized in Figure 2, MWA was present in 0% of individuals (n = 6) with 0 "risk factors", 11% of individuals (n = 19) with 1 "risk factor", 14% of individuals (n = 69) with 2 "risk factors", 18% of individuals (n = 92), with 3 "risk factors", 39% of individuals (n = 56) with 4 "risk factors" and 75% of individuals (n = 4) with all 5 dopaminergic allelic "risk factors".

Finally, MWA frequency was determined in individuals with 0, 1 or 2 allelic "risk factors" vs. individuals with 4 or 5 allelic "risk factors". Individuals with 4 or 5

allelic "risk factors" have a significantly greater (Chi-square = 16.67; $p < 0.00002$) incidence of MWA (42%) than individuals with 0,1 or 2 allelic "risk factors" (13%).

II. Association Between Comorbid Migraine, Anxiety and Depression and DRD2 NcoI Alleles

In epidemiological studies, a clinical diagnosis of migraine significantly increases the risk of comorbid anxiety and depression. However, because all of these diseases are probably multifactorial, the fact that a specific genetic locus may correlate with one of these symptoms does not necessarily imply that it correlates with others. This example tests whether variant forms of dopaminergic genes correlate with depression and anxiety as well as with migraine with aura.

A. METHODS

The data described in this study are derived from a clinical genetic relational database that was developed initially for the genetic analysis of migraine Peroutka & Howell, *Towards Migraine 2000*, (Amsterdam, Elsevier Science B.V., 1996), pp. 35-48. Potential subjects were identified by physician or self-referral. Subjects were evaluated using a semi-structured interview for migraine. Migraine evaluations were conducted by a neurologist and/or trained interviewer. The lifetime presence or absence was determined for each of the criteria in the International Headache Society (IHS) definition of migraine with (MWA) (*Cephalalgia* 8, 1-96 (1988).

A semi-structured interview based on the Structured Clinical Interview for DSM-III-R (SCID) (Spitzer et al., *Arch Gen Psychiatr* 1992;49:624-629), modified to include the criteria of the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) (*Diagnostic and Statistical Manual of Mental Disorders*. Vol. 4. (Washington, DC, American Psychiatric Association, 1994), pp. pp. 317-391 and 393-444) was used to evaluate anxiety and depressive disorders in the same individuals interviewed for

migraine. The interview included questions that were appropriate to establish a DSM-IV-based diagnosis of generalized anxiety disorder (GAD), phobias, panic attacks, panic disorder, obsessive-compulsive disorder (OCD) and major depression. Interviews were performed by physicians or trained psychiatric nurses. Diagnoses required the concurrence of at least 3 physicians.

Genomic DNA was isolated using the Puregene DNA isolation kit (Gentra Systems, Research Triangle Park, North Carolina). Genotyping of the DRD2 *NcoI* polymorphism was performed using previously described primers (Sarkar et al., *Genomics* 11,8-14 (1991). Briefly, 40 ng of genomic DNA was amplified in 10 uL of a solution containing 1x Perkin Elmer PCR amplification buffer, 400 uM each dNTP, 0.5 U *Taq*Gold polymerase (Perkin Elmer, Foster City, CA) and 1 uM primers DRD2.35 (ATCCTGCAGCCATGG) and DRD2.38 (ATTGTCCGGCTTTACC). The enzyme was activated with an initial incubation at 94°C for 10 minutes, followed by 14 cycles of amplification with denaturation at 94°C for 20 seconds, annealing at 63°C for 1 minute, elongation at 72°C for 30 seconds with a decrease of 0.5°C and 3 seconds for each annealing step, and an additional 40 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and elongation at 72°C for 1 minute. After amplification, 10 uL of a solution containing 2x NEB4 buffer and 2 U *NcoI* (New England Biolabs, Beverly, MA) were added directly to the amplification reaction and incubated at 37°C for greater than 4 hours. The digested products were separated on a 1.2% agarose SFR gel (Amresco, Solon, OH). Analysis of the genotype was performed by two individuals blinded to the clinical status.

B. RESULTS

1. Clinical characteristics of individuals in the present study

Direct diagnostic interviews were completed on all individuals in the present study (n = 242). A diagnosis was made only if the individual met DSM or IHS criteria

for the disorders listed in Table 9. If a clear clinical diagnosis could not be made, the subject was not included in any further statistical analyses for that particular disorder. For each of the conditions analyzed, a diagnosis was made in at least 98% of the individuals. In the overall dataset, 55% (134/242) of individuals were diagnosed with at least one of the clinical disorders analyzed. As shown in Table 9, anxiety disorders are the most common diagnosis, being present in 46% (122/242) of the current dataset. Major depression is the single most common diagnosis (i.e., 38%) amongst the group of analyzed disorders in the present study. The incidences of panic attacks (31%) and phobia (29%) are similar. MWA is present in 21% of the individuals. Panic disorder, GAD and OCD are present in less than 20% of the current study group.

TABLE 9

INCIDENCE OF MIGRAINE WITH AURA, ANXIETY DISORDERSAND MAJOR DEPRESSION IN THE INDIVIDUALS (n=242) IN THE PRESENT STUDY

The clinical diagnoses were based on DSM criteria for the anxiety disorders and major depression and on IHS criteria for MWA. If a clear diagnosis could not be made, the individual was "not diagnosed" and was not included in further statistical analyses for the disorder.

	Affected	Unaffected	Not diagnosed
MWA, anxiety or depression	55%	45%	0%
Any anxiety disorder	46%	54%	0%
Major Depression	38%	61%	1%
Panic Attacks	31%	68%	1%
Phobia	29%	69%	2%
Migraine with Aura	21%	79%	0%
Panic Disorder	19%	81%	1%
Generalized Anxiety Disorder	17%	81%	2%
Obsessive Compulsive Disorder	14%	86%	0%

2. Frequency of neuropsychiatric disorders based on DRD2 *Nco* I genotypes

The incidences of the various clinical diagnoses based on DRD2 *Nco*I genotypes is provided in Table 10. A present or past history of MWA, anxiety disorders or major depression is present in 69% of the A1/A1 individuals, 53% of the A1/A2 individuals and 22% of the A2/A2 individuals. The incidence of any of these neuropsychiatric diagnoses is significantly higher in the

A1/A1 individuals when compared to either the A1/A2 individuals (Chi-square=6.53; $p < 0.005$), A2/A2 individuals (Chi-square=15.29; $p < 0.00005$), or the combined A2/any group of individuals (Chi-square=12.72; $p < 0.0002$).

TABLE 10

INCIDENCE OF MIGRAINE WITH AURA, ANXIETY DISORDERS AND MAJOR DEPRESSION IN THE CURRENT DATABASE BASED ON DRD2 *Nco*I GENOTYPES

	A1/A1 (n=131)	A1/A2 (n=93)	A2/A2 (n=18)	Chi-square analysis A1/A1 vs. A2/any	p value
MWA, anxiety or depression	69%	53%	22%	12.72	0.0002
Any anxiety disorder	54%	41%	17%	7.20	0.004
Generalized Anxiety Disorder	23%	11%	11%	6.11	0.007
Major Dep.	45%	33%	17%	5.18	0.01
Panic Attacks	38%	26%	17%	4.96	0.01
Migraine with Aura	26%	17%	6%	4.09	0.02
Phobia	34%	27%	11%	2.60	0.05
Panic Disorder	22%	16%	11%	1.45	n.s.
Obsessive Compulsive Disorder	14%	16%	0%	0.01	n.s.

The presence of an anxiety disorder is significantly more frequent in the A1/A1 individuals than in either the A1/A2 individuals (Chi-square=3.87; $p < 0.02$), A2/A2 individuals (Chi-square=8.92; $p < 0.001$), or the combined A2/any group of individuals (Chi-square=7.20; $p < 0.004$). A similar pattern in seen with GAD. Major depression, panic attacks, MWA and phobia are also all increased significantly in the A1/A1 vs. A2/any individuals (Table 10). Although both panic disorder and OCD are more frequent in the A1/A1 vs. A2/any individuals, the difference does nor reach statistical significance. However, OCD is more frequent in the A1/A1 individuals than in the A2/A2 individuals (Chi-square=2.84; $p < 0.05$).

3. DRD2 *Nco* I allele frequencies in neuropsychiatric disorders in the current study

DRD2 *Nco*I allele frequencies were determined in individuals based on the presence or absence of the neuropsychiatric disorders analyzed in the present study. In individuals with MWA, anxiety disorders and/or major depression, the A1 allele frequency is 0.80 and the A2 allele frequency is 0.20. In individuals who have none of these neuropsychiatric disorders, the A1 allele

frequency is 0.63 and the A2 allele frequency is 0.37. The difference in the DRD2 *Nco*I A1 allele frequencies between these two groups of individuals is highly significant (Chi-square=17.13; $p < 0.00002$).

Table 11

DRD2 *Nco*I ALLELE FREQUENCIES IN INDIVIDUALS WITH OR WITHOUT MIGRAINE WITH AURA, ANXIETY DISORDERS OR MAJOR DEPRESSION IN THE PRESENT STUDY

	A1	A2	Chi-square	p value
MWA, anxiety and/or depression	0.80	0.20	17.13	0.00002
No MWA, anxiety or depression	0.63	0.37		

In conclusion, the present data indicate that MWA, anxiety disorders and major depression are comorbidly associated with allelic variations in the DRD2 gene. As a result, some manifestations of these diseases may constitute a distinct clinical syndrome resulting from a single underlying genetic variation. The clinical recognition that all three disorders are associated with the same genetic variant has significant diagnostic and therapeutic implications.

The foregoing description of the preferred embodiments of the present invention has been presented for purposes of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise form disclosed, and many modifications and variations are possible in light of the above teaching. All publications and patent applications cited herein are incorporated by reference in their entirety to the same extent as if each individual publication or patent application was specifically and individually so denoted.

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*
Clinically Effective DRD2 Anti-Migraine Drugs							
haloperidol	Haldol	psychosis Tourette's ADHD	2-20 mg PO IM	4	alpha = 14 5HT2 = 45	+/+ ++++	McNeil
domperidone	Motilium (Europe)		10-30 mg PO	6	alpha = 74		Janssen (Europe)
prochlorperazine	Compazine	psychosis anxiety antiemetic	10 mg PO IM IV PR	7	alpha = 200		generic (SKB)
chlorpromazine	Thorazine	psychosis anxiety antiemetic MDI hiccups	200-800 mg PO IM IV PR	25	alpha = 4 5HT2 = 19	+++/+++ ++	generic (SKB)
flunarizine	Sibelium (Europe)		10-20 mg PO IV	110			Janssen (Europe)
metoclopramide	Reglan	GE reflux antiemetic gastric stasis	10 mg PO IM IV	160	alpha 10,000		generic (Robins)

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*
Commercially Available But Untested DRD2 Antagonists							
risperidone	Risperdal	psychosis	2-8 mg/day PO	1	alpha 1 < 10 5HT2	++/+++ ++	Janssen *Dr. Jim Gibb*
perphenazine	Trilafon	psychosis antiemetic	8-32 mg PO IM IV	1			Schering
fluphenazine	Prolixin	psychosis	2-20 mg PO IM IV	2	alpha = 8 5HT2 = 25	+/+ ++++	Apothecon
droperidol	Inapsine	antiemetic pre-anes anxiety	2.5-10 mg IM IV	2	alpha = 1 5HT2 = 5	++++	Janssen
trifluoperazine	Stelazine	psychosis anxiety	5-20 mg PO IM IV	3	alpha = 68	+/+ +++	SKB
pimozide	Orap	Tourette's	2-6 mg PO (half life 55 hours)	3	alpha = 78 5HT2 = 25	+/+ +++	Gate Pharmaceuticals
thiothixene	Navane	psychosis	5-30 mg PO IM IV	3	alpha = 11 5HT2 = 36	++/++ +++	Roerig

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*
chlorprothixene	Taractin	psychosis	50-400 mg PO IM IV	8		+++/++ ++	Roche
thioridazine	Mellaril	psychosis anxiety/depression	150-600 mg PO	63	alpha = 7 5HT2 = 63	++++++ +	Sandoz
loxapine	Loxitane	psychosis	60-100 mg PO IM IV	100		+/+ ++	Lederle
molindone	Moban	psychosis	5-250 mg PO	150		+++ ++	Gate Pharmaceuticals
promethazine	Phenergan	antiemetic	25 mg PO IM IV	240		"+++/+++"	generic (Wyeth Ayerst)
clozapine	Clozaril	psychosis	150-450 mg PO	380	alpha = 20 5HT2 = 29	+++/+++ 0 agranulocytosis seizures	Sandoz
trimetho-benzamide	Tigan	antiemetic	200-250 mg	640			generic (SKB)
mesoridazine	Serentil	psychosis EtOH abuse "Psychoneurotic manifestations"	75-300 mg PO IM IV			+++/++ +	Boehringer Ingelheim

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*
thiethyperazine	Torecan	antiemetic	10-30 mg PO IM PR			"+++/+++ ++++"	Roxane Labs
DRD2 Antagonists (In Development)							
amisulpiride	launched	psychosis	25-50 mg (half life = 5 hrs)				Synthelabo
nemonapride	launched (Japan)	psychosis	9-36 mg/day				
sultopride	launched (Belgium)	psychosis					Synthelabo
zotepine	launched	psychosis anxiety			5-HT ant		Fuzisawa
zuclopenthixol	launched (except Us)	psychosis					Lundbeck
sertindole	Serdolect (approved 7/96)	psychosis anxiety	12-20 mg PO		5HT2 ant alpha1 ant D1		Abbott and Lundbeck
olanzepine (? LY 170053)	Zyprex (pre-registration)	psychosis	2.5-15 mg PO		5-HT2 ant		Lilly
quetiapine	Seroquel (Phrase III)	psychosis			5-HT2 ant		Zeneca

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*
ziprasidone (CP-88059)	Phase III	psychosis depression	40-120 mg PO (half-life = 4 hours)		5-HT2A ant (10x D2) 5-HT2C ant 5-HT1D ant 5-HT1A agonist		Pfizer
perospirone	Phase III	psychosis			5-HT2 ant		Sumitomo
iloperidone (HP-873)	Phase III	psychosis		110	alpha=0.4 5-HT2A=6 5-HT2c=43 5-HT6=31 5-HT7=22		HMR
setoperone	Phase III	psychosis			5-HT2 ant		RW Johnson
AD-5423	Phase II	psychosis		15	5HT2=8		Dainippon
mazepertine	Phase II	psychosis		<3	5-HT1A agonist <3 alpha1 ant <3 D3 ant		RW Johnson
bromerguride	Phase II	psychosis			5-HT1A agonist		Schering AG *Lynn Bothello*
1192U90	Phase II	psychosis			5-HT2 ant 5-HT1A agonist		Glaxo Wellcome
carvotroline	Phase I	psychosis	200 mg		5-HT2 ant		Wyeth Ayerst *Jim Barrett*
raciopride	preclinical			3			Schering Plough

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*
zalospirone	preclinical						American Home Products *Jim Barrett*
remoxipride	preclinical			110			Astra
cyclic benzamides	preclinical	psychosis			5-HT2 ant 5-HT1A agonist		Glaxo Wellcome
P 706-A	preclinical	psychosis			5-HT2 ant		Hoechst A.G.
p-9662	preclinical	psychosis			5-HT2 ant		Hoechst-Roussell
ocaperidone	preclinical	psychosis antiemetic	1-2 mg/day		5-HT2 ant		Janssen
LEK-882	preclinical	psychosis			5-HT2 ant 5-HT1A ant D1 agonist		LEK Pharmaceuticals
EMD-56551	preclinical	anxiety			5-HT1a agonist		Merck KGaA
EMD-67478	preclinical	anxiety			5-HT1a agonist		Merck KGaA
EMD-77697	preclinical	psychosis anxiety			5-HT1a agonist		Merck KGaA
NNC-22-0031	preclinical	psychosis			5-HT3 ant alpha1 ant		Novo Nordisk A/S
ORG-10490	preclinical	psychosis			5-HT ant		Organon
ORG-20223	preclinical	psychosis			5-HT1a agonist		Organon

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*
OPC-14597	preclinical						Otsuka
S-16924	preclinical	psychosis			5-HT1A ant 5-HT2 ant D4 ant		Servier
DU-29894	preclinical	psychosis			5-HT1A agonist		Solvay Duphar
umespirone	preclinical	psychosis			5-HT1A agonist		Solvay Duphar
SM-13496	preclinical	psychosis			5-HT2 ant		Sumitomo
SM-9018	preclinical	psychosis					Sumitomo
SDZ-MAR-327	preclinical	psychosis			5-HT1A agonist 5-HT2 ant D1 agonist		Tsububa
ZD-3638	preclinical	psychosis			5-HT2 ant		Zeneca
alentemol	?	psychosis			5-HT2 ant		Parmacia Upjohn
ORG-5222	suspended	psychosis			5-HT ant		Organon
acetophenazine	Tindal (? not in Us)		40-120 mg PO				?
amperoxide	inactive	psychosis					Pharmacia Upjohn/Sandoz
HDC-912	preclinical	psychosis					Sandoz *Available for licensing*

TABLE 1

SPECTRA BIOMEDICAL, INC.
DRD2 Development Program

DRUG	BRAND NAME	INDICATIONS	DOSE	DRD2 AFFINITY	OTHER RECEPTORS	MAJOR SIDE EFFECTS: SED/HYPOTENSION EPS OTHER	COMPANY *CONTACT PERSON*

WHAT IS CLAIMED IS:

1. A method of diagnosing a patient for susceptibility to a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety comprising detecting a variant allele of one or more dopaminergic genes in the patient.

2. The method of claim 1, wherein the one or more dopaminergic genes are selected from a group consisting of DRD1, DRD2, DRD3 and DAT.

3. The method of claim 1, wherein the variant allele is DRD2 NcoI A1, and the presence of homozygous A1 alleles indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A2/A2 alleles.

4. The method of claim 1, wherein the variant allele is DRD1 B1 and the presence of homozygous or heterozygous B1 alleles indicates increased susceptibility to the syndrome relative to homozygous B2 alleles.

5. The method of claim 1, wherein the variant allele is DRD3 A2 and the presence of homozygous A2 alleles indicates increased susceptibility to the syndrome relative to homozygous A1 alleles.

6. The method of claim 1, wherein the variant allele is DAT 10, and the presence of homozygous D10 alleles indicates increased susceptibility to the syndrome relative to heterozygous D10/D9 alleles.

7. The method of claim 2, wherein variant alleles are detected in two or more of the DRD1, DRD2,

DRD3 and DAT, and risk factors associated with the presence of each variant allele detected are combined to indicate susceptibility to the syndrome.

8. A method of treating a patient suffering from the syndrome of claim 1, comprising administering to the patient a therapeutically effective amount of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT.

9. The method of claim 8, wherein the agent lacks specific binding to DRD4 and/or DRD5.

10. The method of claim 8, wherein the agent is any one of the agents shown in Table 1.

11. The method of claim 8, wherein the blood-brain barrier of the patient is impermeable to the agent.

12. The method of claim 8, wherein the agent is administered intravenously, orally or intramuscularly.

13. The method of claim 8, wherein the agent is administered prophylactically.

14. A method of treating a patient suffering from migraine with aura and having homozygous DRD2 NcoI A1 alleles comprising administering to the patient a therapeutically effective amount of an agent that antagonizes binding of dopamine to DRD2.

15. The method of claim 14, further comprising determining that the patient has homozygous NcoI A1 alleles.

16. A method of screening for a drug effective to treat the syndrome of claim 1, comprising determining the capacity of the drug to antagonize binding of dopamine to DRD1, DRD2, DRD3 and/or DAT.

17. The method of claim 16, wherein the drug lacks specific binding to DRD4 and/DRD5.

18. Use of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT for the manufacture of a medicament for use in the treatment of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety in patients having a variant allele of one or more dopaminergic genes.

19. The use according to claim 18, wherein the one or more dopaminergic genes are selected from DRD1, DRD2, DRD3 and DAT.

20. The use according to claim 18 or claim 19, wherein the variant allele is DRD2 NcoI A1 and the presence of homozygous A1 alleles indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A2/A2 alleles.

21. The use according to any of claims 18 to 20, wherein the syndrome is characterized by symptoms of migraine with aura.

22. The use according to any of claims 18-21, wherein the agent is any one of the agents shown in Table 1.

23. A method for determining the suitability of a patient suffering from a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety for treatment with an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT comprising the steps of detecting a variant allele of one or more dopaminergic genes in the patient.

24. The method according to claim 23, wherein the one or more dopaminergic genes are selected from DRD1, DRD2, DRD3 and DAT.

25. The method according to claim 23 or claim 24, wherein the variant allele is DRD2 NcoI A1 and the presence of homozygous A1 alleles indicates increased suitability relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A1/A2 alleles.

26. The method according to any of claims 23 to 25, wherein the syndrome is migraine with aura.

27. The method according to any of claims 23 to 26, wherein the agent is any one of the agents shown in Table 1.

28. Use of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT for the manufacture of a medicament for use in therapy, the therapy comprising the step of:

a) diagnosing a patient for susceptibility to a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety by a method comprising the step of detecting a variant allele of one or more dopaminergic genes in the patient; optionally further comprising the step of

b) administering to the patient a therapeutically effective amount of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT.

29. The use according to claim 28, wherein the one or more dopaminergic genes are selected from DRD1, DRD2, DRD3 and/or DAT.

30. The use according to claim 28 or 29, wherein the variant allele is DRD2 NcoI A1 and the presence of homozygous A1 alleles indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles and the presence of heterozygous A1/A2 alleles indicates increased susceptibility relative to the presence of homozygous A2/A2 alleles.

31. The use according to any of claims 28 to 30, wherein the syndrome is migraine with aura.

32. The use according to any of claims 28 to 31, wherein the agent is any one of the agents shown in Table 1.

33. A diagnostic agent for detecting a variant allele of one or more dopaminergic genes for use in therapy, prophylaxis or diagnosis.

34. An agent according to claim 33, wherein the dopaminergic genes are selected from DRD1, DRD2, DRD3 and DAT.

35. An agent according to claim 33 or 34, wherein the variant allele is DRD2 NcoI A1.

36. An agent according to any of claims 33 to 35 for use in therapy, prophylaxis or diagnosis of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety.

37. An agent according to any of claims 33 to 35 for use in therapy, prophylaxis or diagnosis of migraine with aura.

38. Use of an agent for detecting a variant allele of one or more dopaminergic genes for the

manufacture of a diagnostic for use in therapy, prophylaxis or diagnosis.

39. Use according to claim 38, wherein the dopaminergic genes are selected from DRD1, DRD2, DRD3 and DAT.

40. Use according to claim 38 or claim 39, wherein the variant allele is DRD2 NcoI A1.

41. Use according to any of claims 38 to 40 for therapy, prophylaxis or diagnosis of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety.

42. Use according to any of claims 38 to 41, wherein the syndrome is characterized by symptoms of migraine with aura.

43. Use of an agent that antagonizes binding of dopamine to DRD1, DRD2, DRD3 and/or DAT for the manufacture of a medicament for use in the treatment of a syndrome characterized by symptoms of migraine with aura, depression and/or anxiety associated with the presence of a variant allele of one or more dopaminergic genes.

44. The use according to claim 43, wherein the one or more dopaminergic genes are selected from DRD1, DRD2, DRD3 and DAT.

45. The use according to claim 43 or 44, wherein the variant allele is DRD2 NcoI A1 and the presence of homozygous A1 alleles indicates increased susceptibility to the syndrome relative to the presence of heterozygous A1/A2 alleles, and the presence of heterozygous A1 /A2 alleles indicates increased susceptibility relative to the presence of homozygous A2/A2 alleles.

46. The use according to any of claims 43 to 45, wherein the variant allele is homozygous DRD2 NcoI A1.

47. The use according to any of claims 43 to 46, wherein the syndrome is characterized by symptoms of migraine with aura.

48. The use according to any of claims 43 to 47, wherein the agent is any one of the agents shown in Table 1.

49. The use according to any of claims 43 to 48, wherein the medicament is administered prophylactically.

DOPAMINE RECEPTOR ALLELES AND FREQUENCY OF MIGRAINE WITH AURA

1A: DRD1

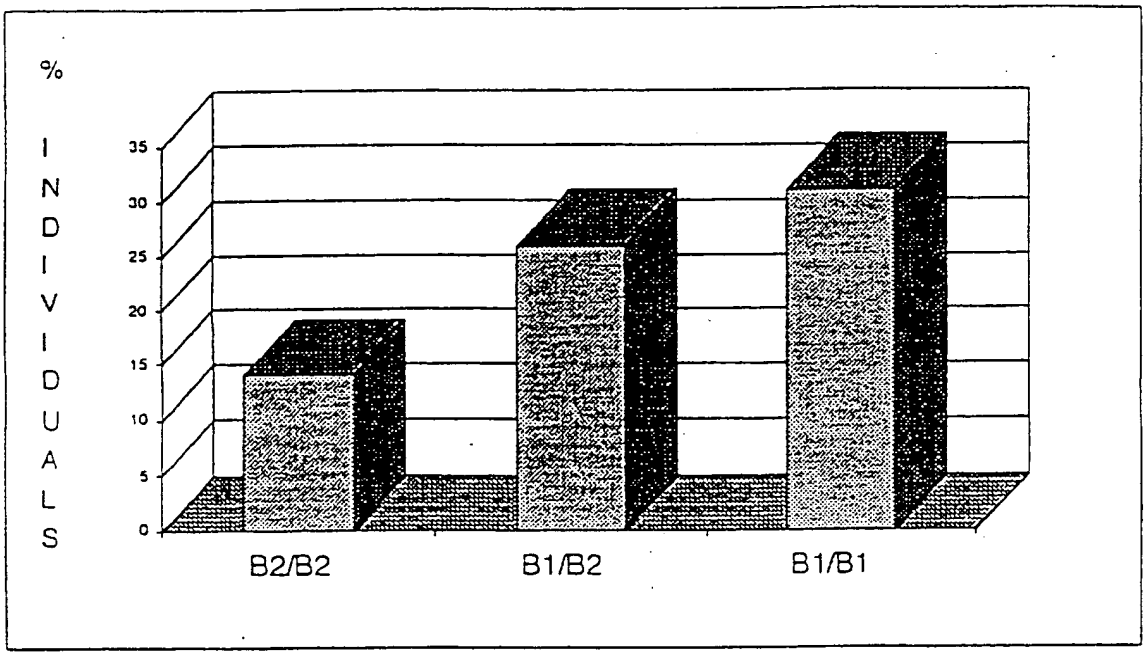

FIG. 1A

DOPAMINE RECEPTOR ALLELES AND FREQUENCY OF MIGRAINE WITH AURA

1B: DRD2

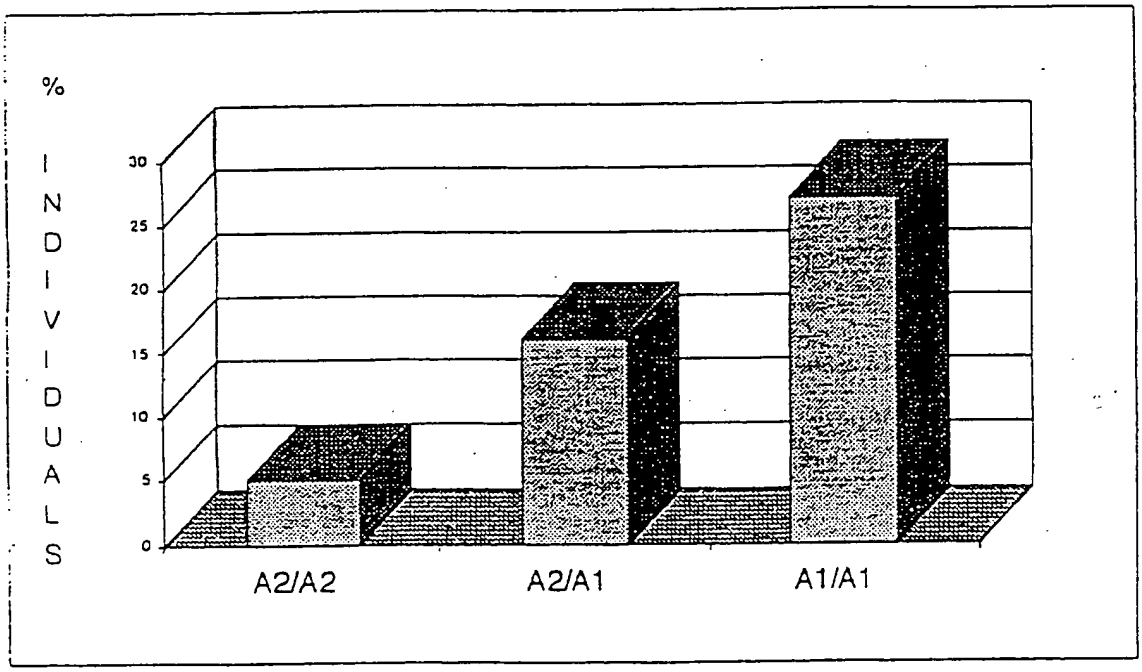

FIG. 1B

DOPAMINE RECEPTOR ALLELES AND FREQUENCY OF MIGRAINE WITH AURA

1C: DRD3

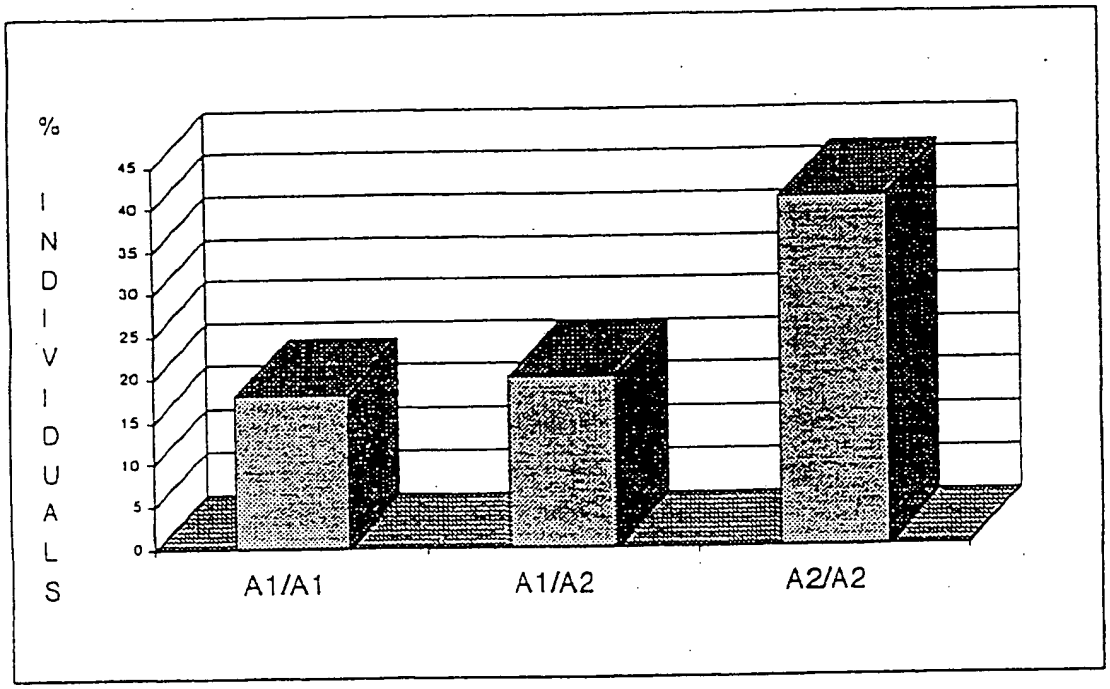

FIG. 1C

DOPAMINE RECEPTOR ALLELES AND FREQUENCY OF MIGRAINE WITH AURA

1D: DAT

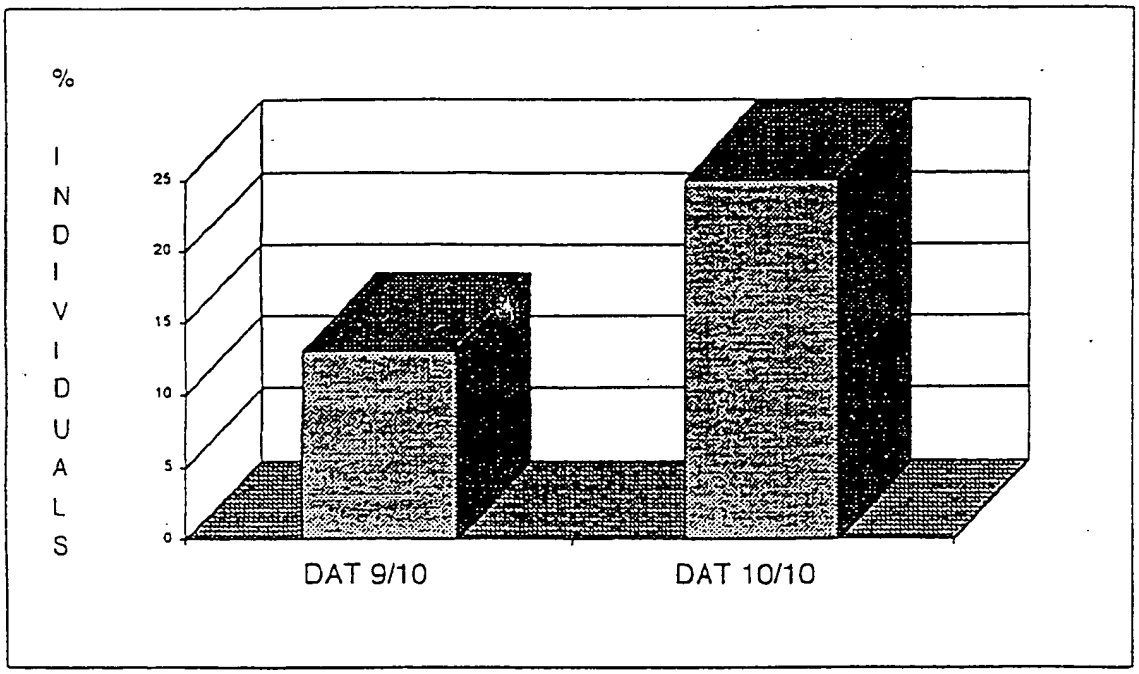

FIG. 1D

INCIDENCE OF MIGRAINE WITH AURA BASED ON THE NUMBER OF ALLELIC RISK FACTORS

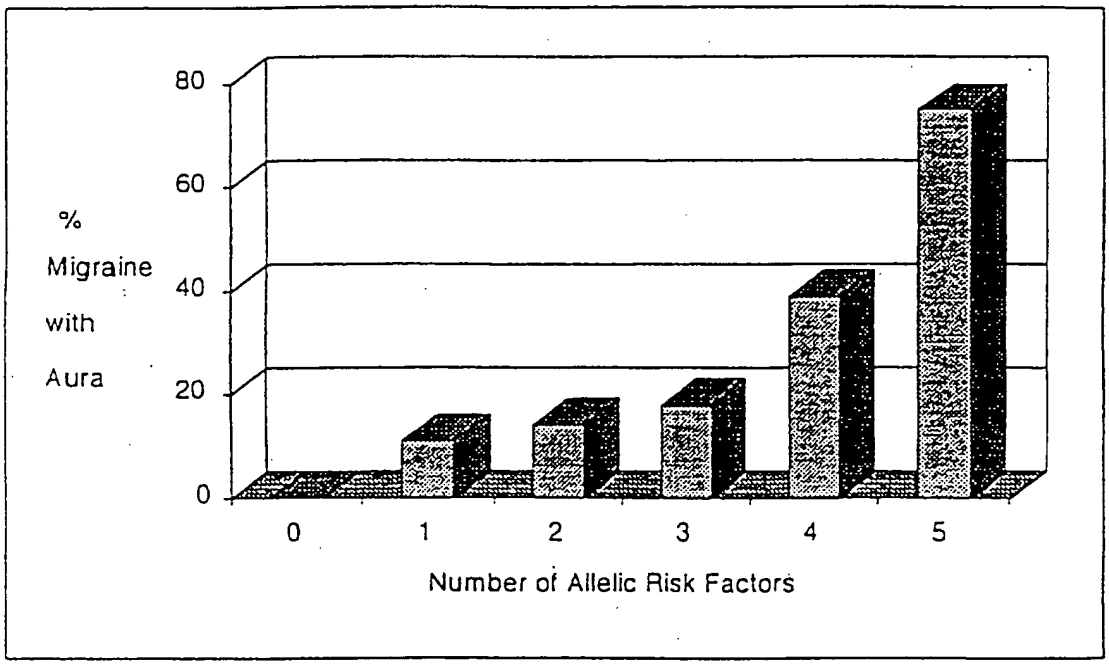

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14830

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/445
US CL : 514/327

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/327

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS AND CAS ONLINE: migraine and aura, depression, anxiety with dopamine?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chem. abstr., Vol. 127, 1997 (Columbus, OH, USA) the abstract No. 147925, PEROUTKA, S.J. 'Clinical Susceptibility to Migraine with Aura is Modified by Dopamine D2 Receptor (DRD2) NcoI Alleles.' Neurology 1997, 49(1), 210-206, see entire abstract.	8-15, 18-22, 28-49

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"B" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

09 OCTOBER 1997

Date of mailing of the international search report

30 OCT 1997

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14830

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-7, 16-17, 23-27
because they relate to subject matter not required to be searched by this Authority, namely:

The claims encompass mental steps such as "diagnosing", etc., which is non-statutory subject matter.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.